

glutathione coated wells. The free cysteines are biotinylated and detected via streptavidin-HRP. We used this assay to study the amount of oxidation and palmitoylation in SNARE proteins (SNAP-25, Syntaxin 1A, and a no cysteine mutant of SNAP-25). Each protein contains 0–4 cysteine residues and results were compared to the background signal obtained from just GST. Reducing agents (Cu^{2+} , Fe^{2+} , Cysteine) and oxidizing agents (cysteine and Zn^{2+}), were used to alter the extent of oxidation/reduction of cysteine residues. Alternatively, reduced cysteines were blocked by reactions with NEM or Palmitoyl-CoA. Except for palmitoylation, all reactions could be driven to near completion during a 10 minute incubation on ice. Palmitoylation (without enzymes) required incubation for 1 hour at RT and high doses of Palmitoyl-CoA to palmitoylate >50% of the cysteine residues. This assay is simple, inexpensive, and relative fast, and should allow greater elucidation of the chemistry of cysteine residues in proteins due to its high resolution.

3282-Pos

Application of Gaussian Network Model to Elucidate Functional Modes of Motion in a Glutamate Transporter

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Glutamate transporters located on the plasma membrane of glia and neurons are responsible for clearing glutamate from the extracellular space and maintaining its concentration below toxic levels. Disruption in glutamate uptake is associated with acute brain injury (ischemia, stroke, seizures) and chronic pathological conditions, including amyotrophic lateral sclerosis, Huntington's disease and Alzheimer's disease. Moreover, glutamate transporters possess a substrate-activated chloride current which may regulate neuronal activity. Understanding the mechanism of transport is therefore, of great physiological and medical importance. High resolution structures of a glutamate transporter homolog (GltPh) from an archaeal species were determined recently (Boduker et al, 2007) allowing for establishing a basis for the structural mechanism of binding and transport at the molecular level. We used the Gaussian Network Model (GNM) for protein dynamics to elucidate the most cooperative modes of motions intrinsically accessible to the homotrimer. A concerted opening and closing of the extracellular vestibule is found to be the most dominant mode of motion near the equilibrium structure. This mode of motion is expected to aid in the substrate binding process of the transport machinery. The movement of the extracellular portions of the protein, towards the center of the aqueous cavity is supported by cross-linking experiments with mutants where residues mutated to cysteine are observed to form disulfide bridges with their counterparts in the neighboring subunit. In the X-ray structure, these residues are more than 40 Å apart. The formation of these disulfide bridges also results in reduced transport of glutamate, suggesting a functional role for the motion induced by the most cooperative GNM mode.

3283-Pos

Simulation of Membrane Sculpting by EFC F-BAR Domain Lattices

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Cells, during cellular morphogenesis, are dynamically sculpted into different compartments by membranes with the help of proteins. The BAR domain is one of the conserved protein domains that is involved in shaping cellular membranes *in vivo*, and is observed to induce tubule formation from liposomes *in vitro*. Previous simulations showed that certain lattice arrangements of N-BAR domains shape membranes into tubules (Yin, Arkhipov, and Schulten, 2009). Here we show, by means of several microsecond coarse-grained simulations of F-BAR domains in different lattice configurations on POPC/POPS membranes, that extended-FCH (EFC) F-BAR domains shape membrane in a fashion similar to what has been seen in N-BAR simulations. The membrane bending property of several F-BAR domain lattice arrangements is characterized, showing that different lattice configurations induce a range of membrane curvatures. A highly detailed view of the dynamic membrane sculpting process by F-BAR domain lattices on a microsecond timescale is obtained. Yin, Y., Arkhipov, A., and Schulten, K. (2009). Simulations of membrane tubulation by lattices of amphiphysin N-BAR domains. *Structure* 17(6), 882-92.

3284-Pos

A Dynamical Model for Heat Shock Protein (HSP) Transcription and its Correlation with HSP70 *in vitro* Experiments

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Heat-shock proteins (HSPs) are well known for their protective mechanism when cells undergo stress conditions such as heat by enhancing protein folding

and mitigating apoptosis. Heat-shock causes the denaturing of proteins to increase, which leads to the aggregation of denatured and functional proteins. On the other hand, cells develop thermotolerance to protect themselves from later exposure to heat shock or other stresses. Once a cell has been exposed to the heat-shock, the fate of the cell depends on the amount of protein denaturation and aggregation. The cell's ability to become tolerant of heat after the heat-shock is one of the most widely studied activating responses experimentally. In the paper, we develop three mechanistic models in a hierarchical fashion that follow a biological pathway from the initial heat-shock to the HSP70:HSF complex that dissociates to HSP70 and HSF, and aim to predict the HSP concentration in a cell. These models allow investigators to simulate the major/important mechanisms in the upregulation of HSP70. This study was performed to create a depiction of the cellular and nuclear events of the HSP70 response to the heat-shock. The data generated from the model were correlated to favorably experimental data under the assumption of time delay.

3285-Pos

Tuning Protein Dynamics by Modulating Solvent Motions through Glassy Matrices, Sol-Gels and Reverse Micelles

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Many proteins can be viewed as nanodevices whose functions are controlled by a complex array of molecular motions. Functionally important protein dynamics pose a difficult challenge for study in that they span orders of magnitude with respect to amplitudes and time scales. One approach to organizing and systematically studying protein dynamics is based on the degree to which the different protein motions are slaved to different categories of solvent motion. This approach is the basis for the solvent slaving model of Frauenfelder and co-workers and our protein dynamic state model. In the present work, we describe how sol-gels, trehalose-derived glasses and reverse micelles can be used as matrices to systematically tune these different categories of protein dynamics through modulation of solvent dynamics and confinement volume. Through this approach we can dissect out the factors that control the activation energies and onset of activation for dynamics in hemeproteins such as hemoglobin and myoglobin that control substrate/ligand diffusion within the protein as well as escape from or entry into the protein from the solvent.

3286-Pos

Structural and Dynamical Behavior of the HIV-1 Nucleocapsid Protein by Molecular Dynamics and Quantum Mechanical Simulations

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The human immunodeficiency virus-type 1 (HIV-1) nucleocapsid (NC) protein is critical for the recognition and packaging of the viral genome and has been a potential target for the development of antiviral drugs. All retroviral NC proteins contain one or two copies of an invariant array of CCHC (Cys-X₂-Cys-X₄-His-X₅-Cys) that are crucial for RNA genome recognition, packaging, and infectivity and have been proposed to function as zinc-binding domains. Although the solution structures of the NC protein with Zinc binding have been determined by experimental studies, the physiological relevance of zinc coordination has not been revealed. Here, we present the structural characteristics of the NCp7 protein with Zn and without Zn ions to investigate the coordination effect in the structural transitions upon inhibitory process by potential drugs by using molecular dynamics simulations with explicit water. For the elucidation of the structural and dynamic properties of the Zn-ligand binding domain in NCp7 protein, quantum mechanical calculations were executed to properly develop the force field for Zn. Upon ligand binding to NCp7 protein, correlation between experimental and computed dissociation constants will be discussed with the mechanistic variations for the ligand-binding and inhibition of NCp7 protein.

3287-Pos

Bio-Mechanical and Binding Effects in Cleavage of Von Willebrand Factor A2 Domain by Adamts13: Molecular Dynamics, Quantum Mechanics and Force Distribution

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ADAMTS13 is a plasma zinc metalloproteinase crucial for primary haemostasis, the enzymatic activity of which is biomechanically controlled. Shear stress-induced partial unfolding of its substrate, the von Willebrand factor (vWF) A2 domain, is essential for efficient cleavage of the target 1605Y-1606M peptide bond. However, the mechanisms of stress-activated vWF A2 recognition and cleavage by ADAMTS13 have yet to be fully resolved.

From our newly developed Molecular Dynamics (MD) based force distribution analysis, we find force to propagate in the cleavage-ready vWF A2 unfolding intermediate such that the target peptide bond is selectively pre-stressed and